ATTACHMENT II

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

APR 03 1997

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Vinclozolin (3rd)

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Acting Section Head
Review Section III
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Budd 3/19/97
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TO: Connie Welch, PM 21
Fungicide/Herbicide Branch
Registration Division (7505C)
and
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THROUGH: Stephanie R. Irene, Ph.D. Stephanie R. Irene 3/31/97
Deputy Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on Jan. 15, 1997 to discuss and re-evaluate the weight-of-the-evidence on vinclozolin with particular reference to its carcinogenic potential, following the Science Advisory Board (SAB) meeting. The CPRC took into consideration *preliminary* findings from the Registrant's re-evaluation of the pathology for the tumors of the ovary and prostate. Based on this re-evaluation, the increases in both ovarian and prostate tumors were no longer statistically significant; however, the increases in prostate tumors were considered by several members to be equivocal. The majority of the CPRC agreed that, based on the revised pathology, vinclozolin should be classified as a Group C - possible human carcinogen. The CPRC recommended that for the purpose of risk characterization, a non-linear approach, Margin of Exposure (MOE), should be used for quantitation of human risk based on hormone-related effects.

Cleared

SUMMARY

At the second Carcinogenicity Peer Review meeting, vinclozolin was classified as Group B2, based on the multiple tumors seen in the rat (benign testicular Leydig cell and prostate adenomas in males, and benign ovarian sex cord stromal tumors in females) at a dose which was not excessively toxic. Hormonal disruption as a basis for the Leydig cell tumors was considered, and the CPRC agreed that a mode of action (antiandrogenic) for these tumors in rodents appears to have been demonstrated. The consensus of the CPRC was that the relevance of Leydig cell tumors to humans could not be dismissed. The CPRC also agreed that hormonal mechanisms for prostate tumors and ovarian sex cord stromal tumors have not yet been developed. There are several chemicals structurally related to vinclozolin also associated with Leydig cell tumors in rats. A non-linear Margin of Exposure (MOE) approach was recommended for estimating risk because the tumors were mainly benign and there was little concern for mutagenicity of vinclozolin.

At the present third meeting on vinclozolin, the CPRC was presented with *preliminary* results of a re-evaluation of the pathology slides from the ovary and prostate of the rat. It was noted that the re-evaluation had not undergone a pathology peer review in accordance with PR-94-5; however, it was decided to provisionally accept these data. Based on these data, the only remaining tumors with increases that were statistically significant were the Leydig cell tumors in the rat; although, some members felt that the increases in prostate tumors were equivocal, but could not be dismissed since there were no prostate tumors in concurrent controls.

The CPRC also considered the recommendations from the October 30, 1996 joint meeting of the FIFRA Scientific Advisory Panel and Science Advisory Board (Panel). The results of this meeting were equivocal and it appeared that a consensus opinion from the panel as a whole was not reached.

The majority of the CPRC agreed that, based on the revised pathology, vinclozolin should be classified as a Group C - possible human carcinogen - and that a non-linear approach (MOE) based on a NOEL for hormone-related effects, should be used for quantitation of human risk. The MOE approach was chosen because the remaining tumors (Leydig cell) were benign at dose levels which were not considered to be excessive, and there was little concern for mutagenicity of vinclozolin.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene

William Burnam

Karl Baetcke

Marion Copley

Yiannakis Ioannou

Hugh Pettigrew

Yin Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Edwin Budd[1] Edwin Budd

Lori Brunsman

Lucas Brennecke[1] (PAT/ORNL) SEE 3a

3. Other Attendees:

Bernice Fisher, Albin Kocialski, Jim Rowe, Joycelyn Stewart, Susan Makris, Kathryn Boyle, David Anderson (HED)

[1]Signature indicates concurrence with pathology report.

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3. Other Attendees:

Bernice Fisher, Albin Kocialski, Jim Rowe, Joycelyn Stewart, Susan Makris, Kathryn Boyle, David Anderson (HED)

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B. Material Reviewed:

The material available for review consisted of the Carcinogenicity Peer Review of Vinclozolin (2nd), preliminary data from the Registrant's re-evaluation of pathology slides from the prostate and ovary of the rat, the report of th Science Advisory Board (SAB), dated December 6, 1996, and other data summaries by Dr. David Anderson. The material reviewed is attached to the file copy of this report.

C. Background Information:

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on August 30, 1995 and April 17, 1996 to consider the carcinogenic potential of vinclozolin. At that time, the majority of the CPRC agreed that vinclozolin should be classified as a Group B2 - probable human carcinogen - and that for the purpose of risk characterization, a non-linear approach - Margin of Exposure (MOE) - should be used for quantification of human risk. This classification was based on statistically significant increased incidences of testicular Leydig cell adenomas and prostate adenomas in male Wistar rats and an increased incidence of ovarian sex cord stromal tumors ($p = 0.053$) in female rats at a dose (500 ppm) which was not excessive. The MOE approach was chosen because the tumors were benign at a dose level which was not considered to be excessive, and there was little concern for mutagenicity of vinclozolin. Mechanistic data for the Leydig cell tumors also provided further support for the use of the MOE approach. For further information, see the memorandum titled "Carcinogenicity Peer Review of Vinclozolin (2nd)", dated September 18, 1996.

On October 30, 1996, the FIFRA Scientific Advisory Panel and Science Advisory Board (Panel) met jointly to review a set of scientific issues posed by EPA relating to vinclozolin and its carcinogenic potential. At the meeting, representatives from EPA presented a review of the carcinogenicity data and issues on vinclozolin. Also at the meeting, representatives from BASF Corporation presented new information, including a re-evaluation of the microscopic slides from the Wistar rat studies, relating to the prostate and ovarian tumors. This information had not previously been submitted to EPA. A general discussion ensued. Following the meeting, the Panel presented its interpretation of the data and its view of the carcinogenic potential of vinclozolin in a report dated December 6, 1996.

Summary of the SAP/SAB Panel Recommendations:

The specific questions posed by EPA and the Panel's response to each question are included in the Panel report, dated December 6, 1996. A summary of the Panel's recommendations is presented below.

The Panel noted that the target tissues of concern (testes, prostate, ovary) are endocrine controlled, with a relatively high incidence of proliferative lesions in controls. Based on the re-evaluation of the prostate and ovarian pathology, the Panel considered the proliferative lesions in these organs to be mostly hyperplasia, with few adenomas and no malignancies. Further, the benign Leydig cell tumors observed in the testes, although significantly increased, were considered to be a common occurrence in the Wistar rat. Based on the re-evaluation of the microslides, the Panel believed there was a significant increase only in testicular Leydig cell adenomas. The Panel concluded that "based on these data, it is far from established that vinclozolin is carcinogenic to the rat. It is not ruled out, however." In reaching this conclusion, it is not clear if the Panel considered the significant increase in testicular cell adenomas (benign) to not require a "carcinogenic" classification for vinclozolin because the observed tumors were benign rather than malignant, of if there was some other reason for the Panel's conclusion.

Noting the increased levels of luteinizing hormone (LH) and serum testosterone in Wistar rats treated chronically with vinclozolin and additional data showing specific inhibition of the testosterone receptors by vinclozolin, and also reduced prostate weights and decreased anogenital distance in other studies, the Panel concluded there is "little doubt that vinclozolin has antiandrogenic action. The increased testosterone levels are likely the factors producing Leydig cell hyperplasia/tumors."

When asked to comment on the Agency's classification of vinclozolin as a B2 - probable human carcinogen - , the Panel stated "we would consider the possibility that vinclozolin is a carcinogen in rats or mice, but the evidence for this is not compelling. The Panel believes that the classification of vinclozolin using the new guidelines would be 'not likely to be a carcinogenic hazard to humans'".

Regarding the method of quantification recommended by the Agency (MOE approach), the Panel agreed with the Agency and stated "the most appropriate method of risk quantification is on a non-linear model, MOE approach based on a NOEL for non-neoplastic effects."

D. **Evaluation of Carcinogenicity Evidence:**

Data previously evaluated by the CPRC are presented in the memorandum dated September 18, 1996, which describes the second peer review meeting held on April 17, 1996. See this memorandum for more detailed information. Data not previously considered by the CPRC are presented below.

1. Pathology Re-evaluation of Ovary and Prostate Tissues from Studies on Wistar Rats Using Vinclozolin as the Test Material

Reference: Capen, C.C., "Pathology peer-review of ovary and prostate; studies with vinclozolin; reg. no. 83 258", consultant to BASF Aktiengesellschaft (Department of Toxicology, Ludwigshafen, Germany), MRID none. 21 pages. Data presented to the FIFRA Scientific Advisory Panel and Science Advisory Board (Panel) joint meeting on October 30, 1996.

a. Experimental Design: Microslides from the ovary and prostate from three studies in Wistar rats with vinclozolin were reviewed independently by Charles C. Capen, DVM, Ph.D. (Ohio State University). The three studies were: 1) 24-month carcinogenicity study in rats, BASF (Germany), Study # 7150375/88105, May 2, 1994, MRID 43254703, 2) 24-month chronic toxicity study in rats, BASF (Germany), Study # 7150375/88026, May 3, 1994, MRID 43254701, and 3) 3-month feeding study in rats, BASF (Germany), Study # 3950375/88116. Dr. Capen and Dr. Gembardt (BASF, Germany), the original study pathologist, then met in Columbus, Ohio, in October, 1996 "to review all microslides on a dual-headed microscope where our histopathologic diagnoses previously had not been in agreement. A consensus was reached on all cases and the pathology data tables generated represent our final diagnoses on the lesions in ovary and prostate glands."

IMPORTANT NOTE: The peer review procedure followed by Drs. Capen and Gembardt was not in accordance with PR Notice 94-5 (August 24, 1994), which sets forth specific procedures to be followed for submission of pathology re-reads to the Agency. In particular, the procedure was deficient in that a Pathology Working Group (PWG) had not been convened to resolve differences in diagnoses between Dr. Capen (peer review pathologist) and Dr Gembardt (study pathologist). Nevertheless, the SAP/SAB Panel did accept and utilize this pathology re-read in its deliberations on the carcinogenic potential of vinclozolin. In considering the recommendations of the Panel, the CPRC also decided to accept and utilize the pathology re-read, but on a tentative basis only pending submission by BASF of an acceptable peer review re-read of the ovary and prostate microslides performed in full accordance with PR Notice 94-5. It is the understanding of the CPRC that BASF intends to do this in the near future. The CPRC further assumed that the pathologic diagnoses in Dr. Capen's report and that by the PWG will not differ significantly. In the interim until an acceptable pathologic re-read is submitted, the evaluation and classification of the carcinogenic potential of vinclozolin reported in this document must be considered tentative.

b. Discussion of Tumor Data:

Ovary

Since age-related ovarian cortical stromal hyperplasia frequently occurs in old rats, the histopathologic criteria in Dr. Capen's report were more clearly defined "to classify 5 different grades of ovarian cortical stromal hyperplasia based upon severity and extent of involvement of the gonad, and to distinguish stromal hyperplasia from benign ovarian sex cord/stromal tumors". Detailed criteria for the 5 grades of ovarian cortical stromal hyperplasia and for benign ovarian sex cord/stromal tumors were presented in the report. At the CPRC meeting, Dr. Lucas Brennecke, pathology consultant to EPA, concurred with the histopathologic criteria used in Dr. Capen's report and the CPRC decided to accept and utilize the re-read of the ovary microslides subject to confirmation of the data, as described previously, by the future submission of an acceptable PWG report by BASF Corp.

Based on the revised criteria for ovarian proliferative lesions, the incidence and graded severity of ovarian cortical stromal hyperplasia and the incidence of benign ovarian sex cord/stromal tumors in the carcinogenicity study (study # 7150375/88105) and the chronic toxicity study (study # 7150375/88026) are presented in Tables 1 and 2 respectively. Using the revised criteria, considerably more hyperplasias and fewer tumors were recorded than reported previously in the original study report.

In the carcinogenicity study, percentage incidences of ovarian cortical stromal hyperplasia were 96%, 96%, 98% and 90% for the 0 (control), 50, 500 and 3000 ppm dose groups respectively. Mean severity scores were 2.5, 2.7, 2.9 and 3.4 for the control and dose groups respectively. In the chronic feeding study, percentage incidences of ovarian cortical stromal hyperplasia were 90%, 95%, 100%, 100% and 100% for the 0 (control), 150, 500, 1500 and 4500 ppm dose groups respectively. Mean severity scores were 2.4, 2.4, 2.8, 3.1 and 4.1 for the control and dose groups respectively. Since ovarian cortical stromal hyperplasia occurred in nearly all female rats in the study by 24 months (percentage incidence in all groups, including the control groups, was 90%-100%), a treatment-related effect on incidence could not be established. The mean severity scores in both studies, however, were dose-related, indicating an effect on this parameter--the mean severity increasing as the dose of vinclozolin increased.

Regarding the benign ovarian sex cord/stromal tumors, percentage incidences in the carcinogenicity study were 4%, 4%, 4% and 10% for the 0 (control), 50, 500 and 3000 ppm dose groups respectively. Percentage incidences in the chronic feeding study were 0%, 0%, 0%, 0% and 5% for the 0 (control), 150, 500, 1500 and 4500 ppm dose groups respectively. The only increased incidence in treated animals in both studies was a slight increase at the highest dose tested (3000 ppm in the carcinogenicity study and 4500 ppm in the chronic toxicity study). These dose levels (3000 ppm and 4500 ppm respectively) had previously been determined by the CPRC to be excessive for evaluating the carcinogenic potential of vinclozolin (see the memorandum describing the 2nd meeting, dated September 18, 1996). It was agreed by the CPRC, based on the revised criteria for the ovarian proliferative lesions, that a treatment-related increased incidence of benign ovarian sex cord/stromal tumors was not observed in either the carcinogenicity or chronic toxicity study at a dose level which was not excessively toxic.

Table 1. Incidence and Graded Severity of Ovarian Cortical Stromal Hyperplasia and Incidence of Benign Ovarian Sex Cord/Stromal Tumors in Carcinogenicity Study (Study # 7150375/88105) with Vinclozolin in Wistar Rats
Based on pathology re-reads in Dr. Capen's report

	0 ppm	50 ppm	500 ppm	3000 ppm
No. exam.	50	49	50	50
Ovarian Cortical Stromal Hyperplasia				
Incidence	48	48	49	45
Percent	(96%)	(96%)	(98%)	(90%)
Mean Severity*	2.5	2.7	2.9	3.4
Benign Ovarian Sex Cord/Stromal Tumor				
Incidence	2	2	2	5
Percent	(4%)	(4%)	(4%)	(10%)

*Range of severity grades: 0 - 5

Table 2. Incidence and Graded Severity of Ovarian Cortical Stromal Hyperplasia and Incidence of Benign Ovarian Sex Cord/Stromal Tumors in Chronic Toxicity Study (Study # 7150375/88026) with Vinclozolin in Wistar Rats
Based on pathology re-reads in Dr. Capen's report

	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
No. exam.	20	20	20	20	20
Ovarian Cortical Stromal Hyperplasia					
Incidence	18	19	20	20	20
Percent	(90%)	(95%)	(100%)	(100%)	(100%)
Mean Severity*	2.4	2.4	2.8	3.1	4.1
Benign Ovarian Sex Cord/Stromal Tumor					
Incidence	0	0	0	0	1
Percent	(0%)	(0%)	(0%)	(0%)	(5%)

*Range of severity grades: 0 - 5

Prostate

Re-evaluation by Dr. Capen of the prostate microslides from the carcinogenicity and chronic toxicity studies using the same histopathologic criteria previously used by Dr. Gembardt (RENI criteria; Harleman, et al., 1996)[3] did not result in any different diagnoses between the two pathologists (see Table 2). In the carcinogenicity study, percentage incidences of focal hyperplasia of the prostate acinar epithelium were 22%, 34%, 50% and 40% for the 0 (control), 50, 500 and 3000 ppm dose groups respectively. The increases at 500 ppm and 3000 ppm were statistically significant ($p < 0.05$). In the chronic feeding study, percentage incidences of focal hyperplasia of the prostate acinar epithelium were 10%, 10%, 17%, 40% and 22% for the 0 (control), 150, 500, 1500 and 4500 ppm dose groups respectively. In neither study was the increased incidence strictly dose-related since the highest incidence in both studies occurred in mid-dose groups. Historical control data for this laboratory, referred to by Dr Capen on p. 4 of his report, was based on 34 chronic studies in Wistar rats (November, 1981 to April, 1995) in which the incidence of focal hyperplasia of the prostate acinar epithelium ranged from 0 to 42% with a mean of 11.4%. Only the 500 ppm dose group in the carcinogenicity study had an incidence (50%) which exceeded the historical control range.

In the carcinogenicity study, percentage incidences of prostate adenomas were 0%, 6%, 14% and 10% for the 0 (control), 50, 500 and 3000 ppm dose groups respectively. The increases at 500 ppm ($p < 0.01$) and 3000 ppm ($p < 0.05$) were statistically significant. In the chronic feeding study, percentage incidences of prostate adenomas were 0%, 10%, 6%, 15% and 6% for the 0 (control), 150, 500, 1500 and 4500 ppm dose groups respectively. Since the highest incidence in both studies occurred in mid-dose groups, in neither study was the increased incidence strictly dose-related. Historical control data for this laboratory, referred to by Dr Capen on p. 4 of his report, was based on 34

[3]Harleman, J.H., Alison, R., Stulin, B., Foley, G.L., Maekawa, A., McConnell, R.F., Quresni, S., Reinm, S., Reznik, G., and Valerio, M.: Male genital system. Vol 8. In: International Classification of Rodent Tumors, Part 1, The Rat. IARC Publication No. 122. U. Mohr, C.C. Capen, D.L. Dungworth, R.A. Griesemer, N. Ito, and V.S. Turusov (eds.). Lyon, France, 1996, in press.

Table 3. Incidence of Proliferative Lesions in Prostate from Carcinogenicity Study (Study # 7150375/88105) and Chronic Toxicity Study (Study # 7150375/88026) with Vinclozolin in Wistar Rats

Based on pathology re-reads using RENI criteria in Dr. Capen's report

Carcinogenicity Study	0 ppm	50 ppm	500 ppm	3000 ppm
No. exam.	48	49	50	50
Focal Hyperplasia, acinar epithelium				
Incidence	11	17	25	20
Percent	(22%)	(34%)	(50%)*	(40%)*
Adenoma, ventral-lateral lobe				
Incidence	0	3	7	5
Percent	(0%)	(6%)	(14%)**	(10%)*

Chronic Tox Study	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
No. exam.	20	20	18	20	18
Focal Hyperplasia, acinar epithelium					
Incidence	2	2	3	8	4
Percent	(10%)	(10%)	(17%)	(40%)	(22%)
Adenoma, ventral-lateral lobe					
Incidence	0	2	1	3	1
Percent	(0%)	(10%)	(6%)	(15%)	(6%)

If *, $p < 0.05$. If **, $p < 0.01$.

chronic studies in Wistar rats (November, 1981 to April, 1995) in which the incidence of prostate adenomas ranged from 0 to 15%. In neither study did the percentage incidence of prostate adenomas in any dose group exceed the historical control range.

Also in Dr. Capen's report was a re-evaluation of selected prostate microslides according to a different set of histopathologic criteria published by Dr. M. C. Bosland (Bosland, 1992)[4], an internationally recognized expert in the pathology of the male reproductive system in rats (see Table 4). Only rats previously diagnosed as having a prostate adenoma (RENI criteria) were included in this re-evaluation. According to Dr. Capen,

[4]Bosland, M.C.: Lesions in the male accessory sex glands and penis. In: Pathobiology of the Aging Rat. Vol 1. U. Mohr, D.L. Dungworth, and C.C. Capen (eds.). ILSI Press, Washington DC, 1992, pp. 443-467.

the previously used more conservative RENI criteria defined prostate adenomas as "an intra-acinar epithelial proliferative lesion obliterating only one acinar lumen, accompanied by some distortion of normal architecture and obliteration of the lumen of the acinus". The Rosland criteria, however, classified "small focal proliferative lesions of prostatic acinar epithelium limited to the involvement of 1 to 3 adjacent alveoli, that do not distort normal alveolar architecture, as **atypical hyperplasia**". At the CPRC meeting, Dr. Lucas Brennecke, pathology consultant to EPA, recommended that the Rosland criteria be used to classify the prostate proliferations observed in the carcinogenicity and chronic toxicity studies on vinclozolin.

In the carcinogenicity study, incidences of atypical hyperplasia were 0, 2, 5 and 3 for the 0 (control), 50, 500 and 3000 ppm dose groups respectively. In the chronic feeding study, incidences of atypical hyperplasia were 0, 0, 0, 3 and 1 for the 0 (control), 150, 500, 1500 and 4500 ppm dose groups respectively. In the carcinogenicity study, incidences of prostate adenomas were 0, 1, 2 and 2 for the 0 (control), 50, 500 and 3000 ppm dose groups respectively. In the chronic feeding study, incidences of prostate adenomas were 0, 2, 1, 0 and 0 for the 0 (control), 150, 500, 1500 and 4500 ppm dose groups respectively.

Recognizing that the proliferative lesions observed in the prostate were focal or multifocal, and that they only developed in older rats with clear evidence of prostate atrophy, it was the consensus of the CPRC, based on the totality of available data on prostate hyperplasia and adenomas, that a treatment-related increased incidence of prostate adenomas was not observed in either the carcinogenicity or chronic toxicity study. This conclusion is subject to confirmation of the data, as described previously, by the future submission of an acceptable PWG report by BASF Corp.

[5]Some members felt that the increases in prostate tumors were equivocal, but could not be dismissed, since there were no prostate tumors in concurrent controls.

Table 4. Incidence of Proliferative Lesions in Prostate from Carcinogenicity Study (Study # 7150375/88105) and Chronic Toxicity Study (Study # 7150375/88026) with Vinclozolin in Wistar Rats

Based on pathology re-reads using Bosland criteria[6] in Dr. Capen's report

Carcinogenicity Study	0 ppm	50 ppm	500 ppm	3000 ppm
No. exam.	0	3	7	5
Atypical Hyperplasia Incidence	0	2	5	3
Adenoma, ventral-lateral lobe Incidence	0	1	2	2

Chronic Tox Study	0 ppm	150 ppm	500 ppm	1500 ppm	4500 ppm
No. exam.	0	2	1	3	1
Atypical Hyperplasia Incidence	0	0	0	3	1
Adenoma, ventral-lateral lobe Incidence	0	2	1	0	0

[6]Only rats previously diagnosed as having a prostate adenoma (RENI criteria) were included in this re-evaluation.

E. Additional Toxicology Data on Vinclozolin:

See the memorandum titled "Carcinogenicity Peer Review of Vinclozolin (2nd)", dated September 18, 1996.

F. Weight of Evidence Considerations:

The CPRC considered the following observations regarding the toxicology of vinclozolin for a weight-of-the-evidence determination on its carcinogenic potential. These observations were directly quoted from the memorandum titled "Carcinogenicity Peer Review of Vinclozolin (2nd)", dated September 18, 1996 (pp. 40-42) except for observations which referred to ovary and prostate tissues in the carcinogenicity and chronic toxicity studies. These observation on ovary and prostate were deleted from the direct quotations (indicated by ---), and replaced by new observations based on the re-evaluation of the ovary and prostate microslides discussed in this document.

1. "Male and female C57/6/JICO mice were fed 0, 15, 150, 3000 or 8000 ppm of vinclozolin, technical for 78-weeks. The highest dose tested (HDT) in the mouse study, 8000 was determined to be excessively high for carcinogenicity testing. This was based on--- statistically significant increases in mortality and body weight gain decrement and reduced relative efficiency of food utilization; liver focal necrosis, bile duct proliferation, pigment storage and lipidosis of the adrenal cortex and obvious endocrine disruption were also observed in males and females. Because of the excessive dosing in this study, the relevance to carcinogenicity in humans of tumors occurring at the HDT was questioned by CPRC.

Hepatocellular carcinoma/adenoma and hemangioma/hemangiosarcoma were statistical significantly increased by pair-wise comparison with controls at 8000 ppm and for increased trend in males and females.

The incidence in testicular Leydig cell hyperplasia in male mice was increased at the 8000 ppm dose level only."

2. "Male and female Wistar rats were fed vinclozolin at 0, 50, 500, or 3000 ppm for 104 weeks (Carcinogenicity Study). Clinical signs of toxicity were observed in both sexes and in males, there was also a 35% body weight decrement accompanied by a reduced food efficiency; in females, there was a 27% reduction in body weight and reduced food efficiency.

There were statistically significant increases in testicular leydig cell --- adenomas in male rats --- at both the mid- and highest doses (500 and 3,000 ppm, respectively). --- Statistically significant increases in uterine adenocarcinomas and adrenal cortical tumors in female rats were found at the highest dose only."

Based on revised criteria for ovarian proliferative lesions, a treatment-related increased incidence of benign ovarian sex cord/stromal tumors was not observed in this study at a dose level which was not excessively toxic. Further, based on a reconsideration of the totality of available evidence on prostate proliferative lesions, it was concluded that a treatment-related increased incidence of prostate adenomas also was not observed in this study. Both of these conclusions are tentative pending confirmation of the data, as described previously, by the future submission of an acceptable PWG report by BASF Corp.

"The majority of the CPRC considered the 3000 ppm dose to be excessively toxic based on the weight gain depressions and other clinical signs. However, it was noted that survival was enhanced in female rats at this dose relative to controls, and survival in males was comparable to that of controls. The CPRC agreed that the next highest dose (500 ppm) was adequate for assessing the carcinogenic potential of vinclozolin in both sexes and considered the tumors which occurred at that dose with statistically significant increases to be relevant: [namely] leydig cell --- adenomas in male rats --- ."

3. "Male and female Wistar rats were fed vinclozolin at 0, 150, 500, 1500 or 4500 ppm (Chronic Study). Body weight gain decrements of >>15% and other clinical signs of toxicity were noted at the highest dose.

Male rats had a statistically significant increase in hepatocellular carcinomas at the highest dose (4500 ppm) and a statistically significant positive trend. Male rats also had statistically significant increases in benign testicular leydig cell tumors at 500, 1500 and 4500 ppm ($p<.01$ at 2 top doses) and a statistically significant positive trend. Female rats administered vinclozolin in this chronic study had statistically significant increases in adrenal combined adenoma/carcinoma ---"tumors" at the highest dose (4500 ppm) and there was a statistically significant positive trend ($p<.01$) for this tumor type."

Based on revised criteria for ovarian proliferative lesions, a treatment-related increased incidence of benign ovarian sex cord/stromal tumors was not observed in this study at a dose level which was not excessively toxic. This conclusion is tentative pending confirmation of the data, as described previously, by the future submission of an acceptable PWG report by BASF Corp.

"The CPRC concluded that the results from this chronic study were consistent with that in the carcinogenicity study, in terms of both the testicular Leydig cell --- tumors and signs of toxicity (body weight gain reductions >>15% and other histological signs). The highest dose (4500 ppm) was considered to be excessively toxic, and the next highest dose (1500 ppm) was considered to be adequate (not excessively toxic)."

4. "From the submitted studies, vinclozolin was not mutagenic in most of the mutagenicity assays with and without activation where applicable. These studies were the Salmonella assay, Chinese hamster ovary (CHO/HGPRT) forward gene mutation assay, mammalian cells in culture cytogenetic assay, rat hepatocyte UDS assay and in the Chinese hamster *in vivo* sister chromatid exchange assay. One study, forward mutation in the mouse lymphoma L5178Y TK+/- 3.72 clone assay, was negative without activation, but equivocal with activation because of test material precipitation. These results indicate little concern for mutagenicity of vinclozolin. The results of a dominant lethal study (HED Doc# 000244) suggest no concern for heritable effects to follow-up at this time."

5. "Vinclozolin and/or its metabolic products are structurally related and related by biological activity to procymidone, flutamide, cyproterone acetate and/or their related metabolic products. All result in androgen deficiency to androgen sensitive organs, cause liver toxicity at high dose levels, and all cause testicular Leydig cell hyperplasia/adenomas and liver carcinomas (liver carcinoma has not been verified for flutamide) at high dose levels. Unlike vinclozolin and procymidone, which have antiandrogenic properties, iprodione administration resulted in decreased plasma androgens possibly by a different mechanism (data in review). Note: procymidone, a weaker antiandrogen than vinclozolin, causes ovarian stromal cell hyperplasia at 2000 ppm. These data were unavailable for consideration by the CPRC.

Vinclozolin and procymidone are negative in a battery of mutagenicity studies and iprodione is positive for unscheduled DNA synthesis (vinclozolin was negative and procymidone was studied and negative for unscheduled DNA synthesis, but the study was inadequate). The literature considers cyproterone acetate a weak mutagen and discusses the possibility that flutamide may be a weak mutagen because a metabolite was found that covalently binds to liver proteins (Fau et al., 1994, abstract).[7] The CPRC indicated that iprodione and procymidone were B2 carcinogens and suggested that flutamide and/or cyproterone acetate may result in liver carcinoma in humans. The evidence taken together (see also Appendix IIIA) was not considered as a basis of support for potential carcinogenicity of vinclozolin for humans."

6. "Hormonal disruption as a basis for the Leydig cell tumors was considered, and the CPRC agreed that a mode of action (antiandrogenic) for these tumors in rodents appears to have been demonstrated (see Appendix of the second Peer Review of vinclozolin for a detailed discussion). The consensus of the CPRC was that the relevance of Leydig cell tumors to humans cannot be dismissed."

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[7] Fau-D, Eugene-D, Berson A, Letteron P, Fromenty-B, Fish-C and Pessayre D. (1994) Toxicity of the antiandrogen flutamide in isolated hepatocyte. J. Pharmacol. Exp. Ther. 269:954-962. Only abstract seen. 4/17

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for vinclozolin.

At the second Carcinogenicity Peer Review meeting on vinclozolin, the majority of the CPRC agreed that vinclozolin should be classified as a Group B2 - probable human carcinogen[7].

At this third meeting, the majority of the CPRC recommended that vinclozolin should be classified as a Group C - possible human carcinogen - and that for the purpose of risk characterization, a non-linear approach (MOE) based on a NOEL for hormone-related effects, should be used for quantitation of human risk. This was based on the Registrant's submission of *preliminary* results[8] of a re-evaluation of the pathology slides from the ovary and prostate of the rat; however, it was decided to provisionally accept these data. Based on these data, the only remaining tumor type with increases that were statistically significant was the Leydig cell tumors in the rat; although some members felt that the increases in prostate tumors were equivocal, but could not be dismissed.

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[7]The proposed revised Guidelines for Carcinogen Risk Assessment (April 10, 1996) were not used to classify vinclozolin, in order to maintain consistency with procymidone and iprodione.

[8]These data had not undergone pathology peer review in accordance with PR-84-5.

Addendum

The toxicology endpoint and dose to be used for non-linear extrapolation and MOE quantitation of potential risk to humans is specified in the "Second Toxicology Endpoint Selection Document" for vinclozolin, dated August 1, 1996. The following is a direct quotation from page 11 of that document.

"**The Selected Endpoint for the Nonlinear Extrapolation and MOE:**
The TESC recommended that the [decreased] epididymal weight from a 2-generation reproduction study (MRID# 42581301 and 43254705) with a NOEL/LOEL = 4.9/30 mg/kg/day be used as the endpoint for the MOE based on the evidence that androgen deprivation (antiandrogen effects) results in both testicular Leydig cell tumors and epididymal weight decrease. Since other tumors were mostly benign, they also would be regulated based on the same endpoint as the testicular Leydig cell tumors."